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Frequencies of SDF-1 Chemokine, CCR-5, and CCR-2 Chemokine Receptor Gene Alleles Conferring Resistance to Human Immunodeficiency Virus Type 1 and AIDS in Kuwaitis

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The frequencies of three mutations conferring resistance to HIV/AIDS were determined in a population sample of native Kuwaitis. The CCR2-64I, SDF1-3′A, and CCR5-m303 mutations were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) tests using restriction endonucleases *Bsa BI, Msp I,* and *Hinc II,* respectively. The frequency of the mutant alleles were: for CCR2-64I, 0.1195 (95% CI 0.0801–0.1694); for SDF1-3′A, 0.2593 (95% CI 0.2024–0.3231), and for CCR5-m303, less than 0.0025. Thus, the CCR2-64I and especially SDF1-3′A mutations are sufficiently common in Arabs and can be used for prognostic genotyping in HIV-infected individuals from the Gulf countries. ***J. Med. Virol. 58:54–58, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: HIV-1; genetic resistance; PCR; allele frequency

INTRODUCTION

Several chemokine receptors have been identified as coreceptors for HIV [Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996; Feng et al., 1996; He et al., 1997; Reeves et al., 1997; Ghorpade et al., 1998]. The degree of HIV coreceptor activity exhibited by different chemokine receptors varies and the "major" HIV-1 coreceptors are CCR5 and CXCR4. HIV strains using these coreceptors are referred to as "R5" and "X4" strains, respectively [Berger et al., 1998]. Importantly, the "R5" strains are responsible for the vast majority of person-to-person transmissions of HIV.

The studies of the role played by chemokines and their receptors in the pathogenesis of AIDS are rapidly progressing. One of the most interesting findings in this field is a discovery of mutations in genes coding for chemokine receptors and their ligands conferring resistance to HIV-1 infection and/or AIDS. Four such mutations have been identified, namely: Δccr-5, CCR5-m303, CCR2-64I, and SDF1-3′A.

The most studied among HIV/AIDS resistance mutations is a 32 nucleotide deletion in the CCR-5 gene, referred to as Δccr-5. The mutation results in truncation of the CCR-5 protein and abrogation of its HIV coreceptor function [Liu et al., 1996; Samson et al., 1996]. The homozygosity for Δccr-5 is highly protective against HIV-1 infection, though the protection is not absolute [Biti et al., 1997; O'Brien et al., 1997; Theodorou et al., 1997]. The Δccr-5 heterozygosity provides partial protection against development of AIDS in HIV-1–infected individuals [Dean et al., 1996; Michael et al., 1997], though there are reports challenging this conclusion [Huang et al., 1996; Garred et al., 1997].

The second mutation in the CCR-5 gene protecting against infection with "R-5" strains of HIV-1 is a single nucleotide polymorphism (T→A substitution at position 303) referred to as m303 or CCR5-m303 [Quillent et al., 1998]. The mutation has been identified in an HIV-exposed–uninfected individual who was a compound heterozygote, i.e., carried the Δccr5 deletion on one chromosome and m303 mutation on another chromosome. The CCR5-m303 mutation results in introduction of premature stop codon in a coding sequence of the first extracellular loop of CCR-5 protein. This leads to the lack of expression of functional CCR-5 protein and abrogation of its HIV coreceptor function.

The CCR2-64I mutation (G→A substitution at position 190 in CCR-2 chemokine receptor gene) results in a valine→isoleucine substitution at position 64 in the CCR-2 protein. The mutation does not protect against HIV-1 infection; however, significantly prolonged AIDS-free survival is observed in CCR-64I–positive

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carriers of HIV-1, even in the heterozygotes [Smith et al., 1997; Kostrikis et al., 1998; Rizzardi et al., 1998].

The SDF1-3′A mutation (also designated as SDF-3′UTR-801G-A) is the single nucleotide polymorphism (G→A substitution at position 801) in 3′-untranslated region of SDF-1 gene encoding chemokine ligand for CXCR4 [Winkler et al., 1998]. The mutation confers significant degree of protection from AIDS, especially at the late-stage disease, as well as some degree of protection from HIV-1 infection demonstrable in exposed but uninfected individuals with extremely high-risk sexual practices.

Global, regional, and ethnic distribution of frequencies of HIV/AIDS protective mutations can vary significantly. Indeed, the Δccr-5 allele has been found to be quite common among individuals of European descent (mutant allele frequency, 0.080–0.12), but it was relatively rare in the Middle East and Asia and absent in Africa [Liu et al., 1996; Samson et al., 1996; Martinson et al., 1997; Voevodin et al., 1997, 1998]. Very little is known about the frequencies of other HIV/AIDS resistance mutations in human populations. Regarding the CCR5-m303 mutation, the only data published are a report of three m303-positive individuals among 209 healthy blood donors from Paris (Quillent et al., 1998). The frequencies of the CCR2-64I allele have been reported for Caucasian Americans (0.098), African Americans (0.151), Hispanic American (0.172), and Asian American (0.250). The frequencies of the SDF1-3′A mutation were also reported for the same population samples: Caucasian Americans (0.211), African Americans (0.057), Hispanic American (0.160), and Asian American (0.257). The frequencies of CCR2-64I and SDF1-3′A alleles in other racial/ethnic groups are not known.

Previously we reported data on the frequency of the Δcr-5 deletion in various ethnic groups residing in Kuwait [Voevodin et al., 1997, 1998]; in this article we present data on the frequencies of the CCR5-m303, CCR2-64I, and SDF1-3′A mutations in Kuwaitis.

MATERIALS AND METHODS

DNA Samples

Blood samples were drawn from 200 healthy, unrelated Kuwaitis. The DNA was extracted from leukocytes using either the “Chelex 100” [Walsh et al., 1991] or “salting out” [Miller et al., 1988] methods.

Polymerase Chain Reaction (PCR) for Detection of the CCR5-m303, CCR2-64I, and SDF1-3′A Mutations

The mutations were detected by PCR-RFLP tests. The amplification conditions were the same for all three tests, namely, standard PCR buffer (10-mM Tris-HCl, pH 8.3; 50-mM KCl, 1.5-mM Mg_2Cl), 50 μM of deoxynucleoside triphosphates, 10 pmoles of each primer, 0.75 unit of AmpliTaq Gold DNA polymerase, and genomic DNA in a total volume of 25 μl. Thermocyling (10 min at 94°C, then 30 cycles at 94°C, 55°C, and 72°C, 30 sec at each temperature) was performed in a Perkin-Elmer system 9600. The primers AV-65 (5′-TTCTTCTTACTGTCCCCTTC-3′) and AV-66 (5′-CTGTGTTTGCGTCTCTCC-3′) were used to amplify a 461-bp fragment of the CCR-5 gene containing the site of the CCR5-m303 mutation. The primers CKR2_1A (5′-TTGTGGGCAACATGATGG-3′) and CKR2_1Z (5′-GAGCCCACAATGGGAGAGT-3′) were used to amplify a 128-bp fragment of the CCR-2 gene, including the site of a single nucleotide polymorphism [Smith et al., 1997]. The primers SDF1-F (5′-CAGTCAACCTGGGCAAAGCC-3′) and SDF1-R (5′-AGCTTTGGTCCTGAGAGTCC-3′) were used to amplify a 302-bp fragment of the SDF-1 gene, including the site of single nucleotide polymorphism [Winkler et al., 1998]. For the detection of the CCR5-m303, CCR2-64I, and SDF1-3′A mutations, the amplified fragments were digested by the restriction endonucleases *Hinc II, Bsa BI,* and *Msp I,* respectively, and then sized by agarose gel electrophoresis. The banding patterns characteristic for different genotypes are presented in Table I.

TABLE I. Size of the Bands Characteristic for Different Genotypes

	Genotype, bp		
Mutation	w/w	mut/w	mut/mut
CCR2-64I	128	128, 110, 18[a]	110, 18[a]
SDF1-3′A	202, 100	302, 202, 100	302
CCR5-m303	400, 61	461, 400, 61	461

[a]The band is not visible in a regular agarose gel.

DNA Sequencing and Analysis

The PCR-amplified fragment of the human SDF-1 genes was sequenced directly using the SDF1-F primer according to the manufacturer’s protocols (Applied Biosystems/Perkin Elmer Foster City, CA) using Dye Terminator Cycle Sequencing Kit with AmpliTaq DNA Polymerase FS and ABI PRISM 310 Genetic Analyzer. To minimize artifacts due to *Taq* polymerase mistakes, samples were prepared for sequencing as a pool of 10 separate identical PCR amplifications of the same target DNA. DNA sequences were aligned and analyzed with help of the DNASIS program (Hitachi).

RESULTS

CCR2-64I

One hundred and thirteen samples were genotyped for the CCR2-64I mutation by PCR-RFLP. Twenty three heterozygotes and two homozygotes were identified, giving the frequency of the CCR2-64I allele in this population sample as 0.1195 (Table II). The distribution of genotypes was in agreement with Hardy-Weinberg equilibrium. A typical result of the CCR2-64I genotyping is presented in Figure 1.

SDF1-3′A

Among 108 individuals genotyped for the SDF1-3′A mutation, 42 heterozygotes and 7 homozygotes were

TABLE II. Frequencies of HIV/AIDS Resistance Mutations in Kuwaiti Population

Mutations	Number of subjects	Number of chromosomes	Number of heterozygotes	Number of homozygotes	Allele frequencies
CCR5-m303	200	400	0	0	<0.0025
CCR2-64I	113	226	23	2	0.1195 (0.0801–0.1694)[a]
SDF1-3′A	108	216	42	7	0.2593 (0.2024–0.3231)[a]

[a]95% confidence interval.

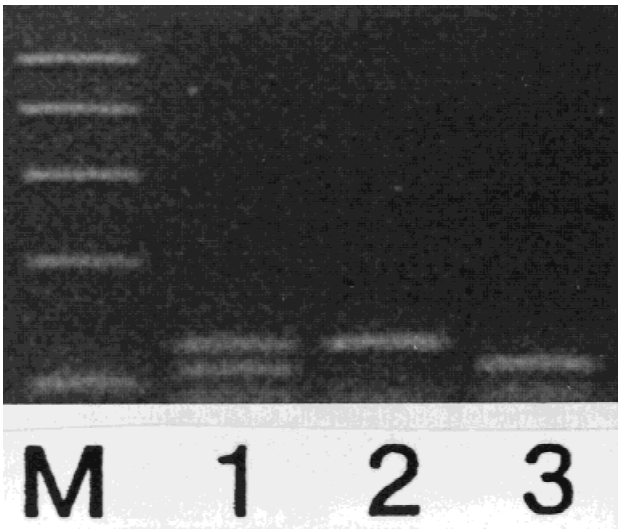

Fig. 1. M, markers (100, 200, 300, 400, and 500bp); 1, CCR2-64I/w heterozygote (CCR2-64I allele band 110 bp, wild-type allele band 128 bp); 2, w/w homozygote; 4, CCR2-64I homozygote.

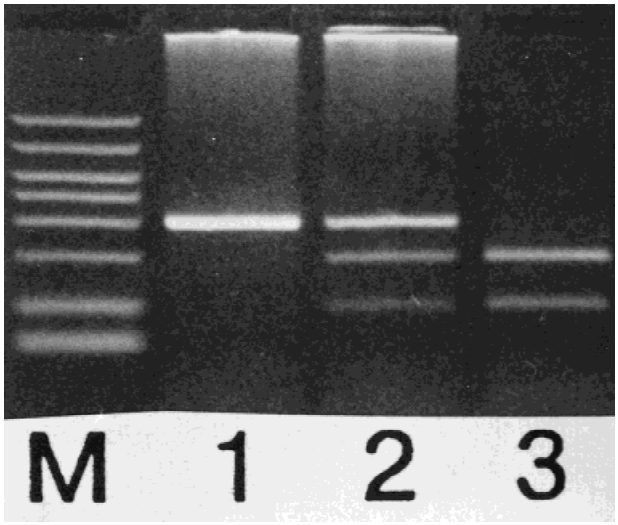

Fig. 2. M, markers (50, 100, 200, 300, 400, 500, 700, and 1,000 bp); 1, SDF1-3'A homozygote; 2, SDF1-3′A/w heterozygote (SDF1-3′A allele band 302 bp, wild-type allele bands 202 and 100 bp); 4, w/w homozygote.

identified, giving the frequency of the SDF1-3′A allele in this population sample as 0.2593 (Table II). The distribution of genotypes was in agreement with Hardy-Weinberg equilibrium. Typical result of the genotyping is presented in Figure 2. It has to be noted that if AmpliTaq DNA polymerase was used instead of AmpliTaq Gold DNA polymerase, a nonspecific band slightly larger than the specific one was observed. The presence of this band can lead to the genotyping errors—false attribution of heterozygous genotype in cases that are wild-type homozygotes. Another potential source of genotyping errors is due to the fact that identification of SDF1-3′A homozygotes is based on the lack of the site for the restriction endonuclease. Thus, the presence of inhibitor of the restriction enzyme could result in false identification of SDF1-3′A homozygotes. Although this possibility was highly unlikely, we confirmed the genotype of two homozygous samples by direct sequencing of PCR products. As expected in both cases, A nucleotide was present at position 801.

CCR5-m303

Among 200 samples tested for the CCR5-m303, no positive was found (Table II). A typical result of CCR5-m303 genotyping is presented in Figure 3. Taking into account that the presence of the site for the restriction endonuclease *Hinc II* is a feature of the wild-type allele, the possibility of genotyping errors was excluded. It is of interest that the test used for detection of the CCR5-m303 mutation allows simultaneous detection of the Δccr-5 deletion (Fig. 3, lane 3).

DISCUSSION

All known genetic determinants of HIV/AIDS resistance are located in either chemokine receptor or chemokine genes. For the Δccr-5 and CCR5-m303 mutations, the mechanism of resistance is quite clear: the mutations result in truncation of the CCR-5 protein and abrogation of its HIV coreceptor function for R-5 strains of HIV-1.

The situation is more complex for the CCR2-64I mutation. Indeed, though some HIV coreceptor activity has been attributed to CCR-2, its significance in this respect is minor as compared to CCR5 and CXCR4. In addition, the CCR2-64I substitution is located in a transmembrane domain of CCR-2 protein, which is not a part of the HIV-binding site. Thus, most probably, the mechanism of protective action of the CCR2-64I mutation is indirect. In this respect, an interesting hypothesis has recently been suggested by Kostrikis et al. [1998]. These authors demonstrated the presence of

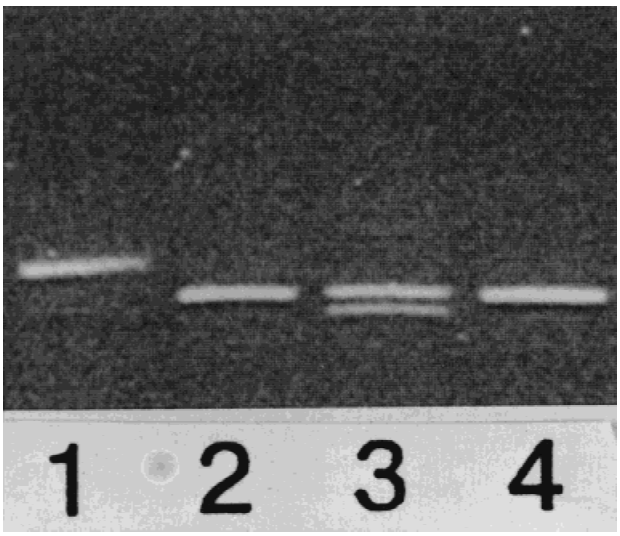

Fig. 3. 1, undigested sample (equivalent of CCR5-m303 homozygote); 2 and 4, w/w homozygotes; 3, w/w homozygote/Δccr5 heterozygote.

the polymorphism in a regulatory region of the CCR-5 gene (C→T substitution at position 59653, GenBank Accession Number U95626), which was in complete linkage disequilibrium with CCR2-64I. If to suggest that this polymorphism can suppress the expression of the CCR-5 protein, then the actual determinant of the CCR2-64I–associated resistance could be the CCR-5/C→T 59653 substitution, while closely linked CCR2-64I (both CCR-2 and CCR-5 genes are located in close proximity on chromosome 3) is only a "tracking" marker.

The protective effect of the SDF1-3′A mutation is recessive, i.e., it is observed only in homozygotes and no difference is apparent in this respect between wild-type homozygotes and heterozygotes. The extent of protection in SDF1-3′A homozygotes is approximately twice as strong as that conferred by either Δccr-5 or CCR2-64I. The mechanism of the protective effect remains unknown. At the same time, a reasonably plausible hypothesis has been suggested. It implies that the 3′ untranslated region of the SDF-1 gene serves as a target for *cis*-acting factors upregulating expression of SDF-1 protein. As a result more of the protein is available to bind CXCR4, the receptor becomes less available for "X4" and "X4-R5" T-tropic strains of HIV-1, and the emergence of more virulent strains characteristic of the symptomatic stage of HIV-1 infection is postponed.

One of the most obvious questions in the framework of the problem of genetic resistance to HIV/AIDS is how frequent the relevant mutations in different racial and ethnic groups are. On the one hand, knowledge of mutation frequencies is essential for gaining insights about their origin as well as the nature of selective factors responsible for accumulation of these genetic polymorphisms in human populations. On the other hand, such information might be helpful in prognosing dynamics of HIV/AIDS epidemics as well as in estimating the cost effectiveness of HIV/AIDS resistance genotyping in various ethnic groups.

Most of the information currently available in this field is related to the Δccr-5 mutation [Christodoulou et al., 1997; Martinson et al., 1997; Voevodin et al., 1997, 1998; Libert et al., 1998]. As was mentioned above, very little is known regarding the frequencies of three other known HIV/AIDS resistance mutations. The aim of this study was to obtain such information for the Kuwaiti population. The native Kuwaitis are descendants of the settlers from the northeastern and central Arabian tribes (Bani Khalid and Utub) and to a lesser extent migrants from Iran and southern Iraq. Thus, the frequencies of HIV/AIDS resistance mutations in our sample is to a certain extent representative of various Middle Eastern populations.

We have shown that the frequencies of the CCR2-64I and SDF1-3′A mutations in Kuwaitis are not significantly different from those in Europeans. These frequencies are sufficiently high to justify genotyping of HIV-positive Kuwaitis (and other Gulf Arabs) for prognostic purposes. Indeed, it can be estimated that approximately 25% of HIV-positive Kuwaitis carry at least one of the three HIV/AIDS resistance mutations (Δccr-5, CCR2-64I, and SDF1-3′A). On the other hand, the CCR5-m303 mutation is either very rare or absent in the Kuwaiti population.

The nature of selective factors contributing to such an extensive accumulation of the SDF-3′A and CCR2-64I mutations in human populations remains unknown. This provides fertile ground for speculations. The most obvious possibility is a selective advantage provided by the mutations with respect to the resistance to infectious diseases that might have had a great impact on survival of prehistoric human populations. Unfortunately, multiple hypothesis possible in this framework are very difficult to test. Nevertheless, the task is worth pursuing.

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